# A STUDY OF THE LEPTOSPIRAL CARRIER STATE OF RATS

An abstract of a Thesis by
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The problem. The Common Norway rat (Rattus norvegicus) is a known carrier and shedder of Leptospira of the serotype icterohaemorrhagiae. This investigation was done to determine if the Des Moines wild rat population was infected with L. icterohaemorrhagiae. The virulence of any isolate was to be tested in laboratory hamsters and rats.

Procedure. Wild Des Moines rats were captured and euthanized, weighed, sexed, and measured for total length. Various tissues were cultured for isolation of infecting leptospires. Leptospiral antibodies were determined from serum samples. Histological examination of rat kidney tissue was done. The $LD_{50}$ value of the wild isolate was determined in laboratory hamsters. Laboratory rats were challenged with a high and low dose of the wild isolate, and then monitored for a serologic and histologic response.

Findings. From the 14 infected wild rats, positive leptospiral isolations were made in 100% of the kidney tissues and 57% of the urine. Eleven of the 14 infected wild rats had antibody titers against L. icterohaemorrhagiae ranging from 1:80 to 1:1000. Renal involvement was observed histologically in infected wild rats. The $LD_{50}$ titer in hamsters was determined to be $10^{5.3}$/ml. Laboratory rats challenged with a high concentration of the wild isolate responded with a titer of 1:80.

Conclusion. Eighteen percent of the wild rat population sampled was infected with leptospires. The microscopic agglutination test determined the isolate to be a member of the icterohaemorrhagiae serotype. Immature wild rats (under 200 grams) were not infected with leptospires. The wild isolate was found to be lethal to weanling hamsters.

Recommendations. Larger samples of the Des Moines wild rat population are needed in order to determine whether the rats infected with leptospires are localized or dispersed throughout the city. A larger sample may also show evidence of leptospiral serotypes other than icterohaemorrhagiae. The immunity from L. icterohaemorrhagiae in young wild and laboratory rats should be studied further to determine the cause of immunity.

# A STUDY OF THE LEPTOSPIRAL CARRIER STATE OF RATS

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David Scott Sandblom

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# INTRODUCTION AND REVIEW OF THE LITERATURE

Weils disease has been recognized as a clincial disease in humans since 1886 (Vander Hoeden, 1958). The etiological agent remained a mystery until 1915 when almost simultaneously in Japan (Inada and Ido, 1915), and in Germany (Uhlenhuth and Fromme, 1915), the infective agent was found to be a spirochete, Spirocheta icterohaemorrhagiae. In 1917, Noguchi described the organism as being different from all other spirochetes. He felt the differences warranted it being placed in a new genus. The name Leptospira was suggested and adopted (Noguchi, 1917). The causative agent of Weils disease is now referred to as Leptospira icterohaemorrhagiae.

Clinical progression of Leptospirosis in Humans. Leptospirosis in humans is mainly an occupationally acquired disease. People working with animals or in rat-infested areas run the greatest risk of exposure. Some of the most commonly infected workers include; veterinarians, dairymen, swineherders, abattor workers, miners, and fish and poultry processors.

The clinical symptoms of leptospirosis in humans are variable, ranging from mild subclinical disease to icteric disease with severe kidney and liver involvement. The incubation period ranges from 3 to 30 days, but most commonly lasts 10 to 12 days. Leptospiremia occurs at the onset of

disease and usually lasts about 7 days. During this time leptospires can also be found in the cerebrospinal fluid (Alexander, 1974). Antibodies appear by the 6th to 10th day, reaching maximal titers by the 3rd or 4th week of disease. Thereafter antibody levels slowly recede, but may be detectable for many years (Wolf, 1954).

Soon after the discovery of the cause of Weils disease in humans, the same infection was discovered in wild rats. In 1916, Leptospira icterohaemorrhagiae was discovered in a great number of Rattus norvegicus (the common Norway rat) by Ido et al. (1917).

Rattus norvegicus has long since been established as the natural vector for leptospires of the icterohaemorrhagiae serotype. After a long sojourn in this species of host its virulence for these animals has been so greatly reduced that the organisms cause the rat no apparent disease (Noguchi, 1917).

L. icterohaemorrhagiae has been isolated from wild rats trapped in cities all over the U.S. as well as world wide. The distribution of the organism goes hand in hand with the geographic distribution of the common rat. In the absence of rats, there is little chance for the propagation and transmission of L. icterohaemorrhagiae (Vander Hoeden, 1958).

Clinical progression of leptospires in the rat. Upon infection of the rat with leptospires, they are rapidly

cleared from the blood stream. They soon find their way to the kidneys, where they become nestled away in the lumina of the convoluted tubules (Lewis, 1942). Here the leptospires carry on a saprophytic existence, generally not influencing the state of health of their carriers (Vander Hoeden, 1958; Ido et al., 1917; Noguchi, 1917). Once infected, rats are usually carriers of leptospires for the rest of their life (Vander Hoeden, 1958; Alexander, 1974).

One of the most confusing, but important, aspects of natural carriers of leptospires is the fact that carrier rats have a very low antibody titer, if any, to the organisms isolated from their kidneys and urine (Babudieri, 1958; Tabel and Karstad, 1967; Broom, 1958; Fuzi and Csoka, 1962). However, upon simulation of "natural" infection in laboratory animals, high titers of long duration to infecting leptospires have been demonstrated (Birnbaum et al., 1972b). Experimentally, it has been shown that the ability to acquire a carrier state in the newborn is dependent upon the immune state of the mother. Mothers with high antibody titers to leptospires transfer immunity to their young, making them unsusceptable for long periods of time to disease produced by homologous leptospires (Birnbaum et al., 1972b).

Of comparable interest is the hypothesis that, due to low immunocompetency, young animals, naturally-infected with low doses of leptospires, have low antibody titers. When the animals reach reproductive age, this results in the inability

to confer passive immunity to offspring. Female animals which are infected at an immunocompetent stage in their development, were unable to transfer the disease to their offspring because the former possessed high titers which were transferred to the newborn as passive protection of long duration (Birnbaum et al., 1972b).

Concurrently, these two theories could account for the observations that some carrier animals possess antibodies and some do not (Birnbaum et al., 1972a). Further research has supported the conclusion of transfer of passive immunity of long duration and has shown that carriership exists among animals two generations apart (Birnbaum et al., 1972b).

Serologic and Diagnostic Methods. Since leptospires can usually be isolated from blood only during the acute phase of the disease, and since such isolations, like animal inoculations, may require several weeks, laboratory diagnosis frequently depends upon demonstrating antibodies in the hosts serum.

A variety of tests have been developed for the serodiagnosis of leptospirosis. Probably the most widely used has been the microscopic agglutination (MA) test with live antigens (Gochenour et al., 1958). Even though this procedure is time-consuming, hazardous and the sensitivity of the living antigen is difficult to standardize, it is still considered the reference test for evaluating other tests. A modified semi-micro method (Sulzer and Jones, 1973), and a

microtechnique (Galton et al., 1965) for the MA test are being used in many laboratories, both of which save much time and material. A modification of the microtechnique (Cole e al., 1973) proved to be most beneficial, because the serological test can be carried out in a microtiter culture plate which contains flat-bottom wells. The results of the test in each well can be easily interpreted by either placing a drop of the mixture on a glass microscope slide or by examining the well contents with the use of a long-working-distance objective on a darkfield microscope.

Several macroscopic agglutination tests have been developed (Stoenner, 1954; Howarth, 1956; Galton et al., 1958) which are easily and rapidly performed. They are of limited use and only of value in screening. Other tests that have been employed in the serologic diagnosis of leptospires include a leptospiral complement-fixation test (Randall et al., 1949), the erythrocyte sensitizing substance test (Chang et al., 1957), and the hemolytic test (Cox, 1955). These tests tend to be mostly genus specific and require fewer antigens to detect leptospiral antibody. However, they are very difficult to perform, and are not practical for small laboratory situations.

Direct Demonstration Methods. The morphologic appearance of leptospires is basically the same for all members of the genus Leptospira. They appear as slender thread-like

organisms, about 0.1μm in diameter and from 6μm to 12μm long, although there has been some exceptions that were 30μm to 40μm. The organism is tightly coiled on its long axis, and the ends are usually bent like a hook. Active young leptospires rotate rapidly on their long axis.

Electron micrographs have revealed an axial filament running the entire length of the organism (Breese et al., 1952; Swain, 1955; Simpson and White, 1961). Leptospires are highly motile and capable of passing through 0.22 micron membrane filters. Leptospires cannot be seen in wet preparations by light-field microscopy, but are readily observed under dark-field microscopy. They stain poorly with the usual bacterial stains. They can, however, be readily stained by several different silver impregnation techniques (Bridges and Luna, 1957; Kerr, 1938; Sulzer and Jones, 1974).

Dark-field examinations of blood and urine frequently results in failure or in misdiagnosis, so it should never be used as the only diagnostic test. Silver staining is most useful in demonstrating leptospires in post mortem tissue sections.

Histological Localizations; Liver Pathology. Upon infection, pathogenic leptospires invade the blood stream and multiply, causing a leptospiremia. During this systemic phase of the disease the leptospires invade most organs of the body. The largest numbers of leptospires were seen in the liver from the 4th to the 7th postinfection day in

hamsters by Miller and Wilson (1966). Leptospires are known to penetrate the sinusoidal wall of the liver, with subsequent localizations in the space of Disse, between and within parenchymal cells. Disruption of parenchymal cells results in disruption of bile canaliculi, causing a release of bile throughout the disrupted areas of the liver and ultimately into the systemic circulation. This release of bile is thought to be in part responsible for the jaundice seen in many animal species during the acute stage of disease (Miller and Wilson, 1966). The known lytic effects of bile on leptospires suggests the role of bile in elimination of leptospires from the liver. Antibodies also would have some effect on the leptospires, but, because of the low titers in the early acute stages of infection, it is likely that bile plays a more active role in destroying the organisms. The majority of the organisms are cleared from the liver by day seven of infection, but some residual organisms may persist for up to 100 days postinfection. The liver is, however, ultimately cleared of all leptospires (Miller and Wilson, 1966).

Histological Localizations; Kidney Pathology. Leptospires are commonly seen in the intertubular capillaries of the renal cortex of the hamster during leptospiremia. Damage was noted by Miller and Wilson (1967) to the endothelial cells and rupture of the capillary walls was often seen whether or not there were leptospires present in hamster kidneys. Leptospires can be found throughout the interstitial tissue

during the acute stage of infection. The proximal convoluted tubule cells are normally involved, and only occasionally are leptospires seen in the distal tubule cells.

Damage of the proximal tubule cells is considerable during the acute stage of the disease. Cellular changes range from swelling to frank necrosis with sloughing of cellular material in the tubular lumen. There is a marked decrease in the number of leptospires in the interstitial tissue at the end of the acute stage of infection. Intracellular leptospires are most prevalent between the 8th and 12th postinfection days (Miller and Wilson, 1967), which coincides with the finding of increased numbers of leptospires in the urine. In the chronic stage of infection, most leptospires are found in the lumin of the proximal tubules ready to be shed in the urine.

Leptospires in the capillaries of the interstitial tissue are thought to escape through the ruptured walls along with erythrocytes and other blood components. The accumulation of these elements in the narrow interstitial spaces provides an excellent medium for the growth and concentration of leptospires seen here during the acute stage of infection (Miller and Wilson, 1967).

Chronic nephritis occurs in prolonged kidney infections. This nephritis is characterized by interstitial nephritis with marked infiltration of lymphocytes, plasma cells, and fibrous connective tissue. The glomerular lesions are

primarily a thickening of the Bowmans capsule and atrophy of affected glomeruli (Hanson, 1976). Agglutinins have been detected in the urine along with leptospires although the sera may contain no agglutinins (Faine, 1963).

The purpose of this study was to determine the incidence of leptospires found in the wild rat population of Des Moines. Once isolated, the "wild type" leptospires were serologically identified. Virulence of the "wild type" leptospires was determined by the use of 21 day old hamsters. A model of infection was set up using albino laboratory rats. The course of disease was followed by monitoring immunological and pathological changes seen in the laboratory rat.

# MATERIALS AND METHODS

## Trapping Rats

Wild Des Moines rats were trapped in live box traps (Havahart) placed in rodent runways and around burrow entrances, both at the Des Moines Children's Zoo and at a local hog buying company. Bait consisted of peanut butter, crackers, and molasses. The traps were checked and reset every morning for a period of five months. Trapped rats were transferred to a holding cage for transport to the laboratory.

At the laboratory, the wild rats were transferred to an anasthesia chamber (10 gallon aquarium). The rats were then euthanized by chloroform over-dose, and immediately processed as described in the following section.

## Isolation of Leptospires

Immediately following euthanization, each wild rat was sexed, weighed, and measured. A 70% alcohol solution was used to rinse the rats in order to reduce the number of external contaminants.

Up to 5 ml of blood was drawn from each rat by means of a cardiac puncture with a 25 gauge needle on a 5 ml syringe. A few drops of the fresh blood from each rat were put into individual tubes of Ellinghausen's semi-solid leptospiral culture media (Ellinghausen and McCullough, 1965) and given four weeks to show bacterial growth. The remaining

blood collected from each wild rat was put into a screw-capped tube and allowed to clot at 4°C. The serum was then pipetted to small viles and frozen for future serologic evaluation.

A mid-sagittal, intraperitoneal incision was made on the ventral side of each wild rat. The incision ran from the manubrium sterni to the pubic area. Special care was taken so that the bladder and ureters were not disturbed. If urine was visible in the bladder, a bladder tap was performed using a 27 gauge needle on a 5 ml syringe. A few drops of urine were then put into individual tubes of Ellinghausen's semi-solid culture media. The remaining urine was diluted with sterile phospate-buffered saline (PBS) in ten-fold dilutions from $10^{-1}$ through $10^{-3}$. One ml of each dilution was inoculated into individual tubes of Ellinghausen's semi-solid culture media. The cultures were then incubated at 29°C for four weeks.

Wild rat kidney samples were obtained by removing one of the kidneys from each rat and placing half of it in the barrel of a 5 ml syringe (without needle). The syringe plunger was replaced and the tissue was forced out through the syringe. The macerated tissue was caught in a tube containing a 10 ml of sterile PBS. The kidney tissues were diluted and cultured using the same procedure as previously described for urine cultures. The other half of the kidneys

were placed in individual tubes containing 10% formalin in PBS, and saved for possible histologic examination.

The top of the skull of each wild rat was removed and a section of brain tissue was taken. The brain tissue was then cultured using the same procedure as outlined previously for kidney tissue.

All culture tubes were viewed daily for bacterial growth. If growth was evident to the unaided eye, leptospiral morphology was verified by darkfield microscopy. Once leptospiral morphology was verified, fresh tubes of Ellinghausen's semi-solid culture media were inoculated with the isolates. These stock cultures were incubated at 29°C for five to seven days, and then stored at 20°C.

Serology

A Coleman photonephelometer was adjusted to 50.5 nephlos (25 nephlos equal $2x10^6$ organisms/ml.) using a titanium dioxide standard (National Animal Disease Center). Each of five serotypes of live antigens (canicola, icteroheamorrhagiae, grippotyphosa, ballum, and pomona) were diluted to a standard concentration of 25 nephlos, as measured by the nephelometer.

Each of the viles of frozen wild rat blood serum was thawed and then diluted 1:5 in PBS. Each solution was then titrated with the five live antigens using the microscopic agglutination test (MAT), (Cole et al., 1973).

Virulence of Wild Leptospiral Isolate

A challenge culture was prepared by inoculation of a tube of Ellinghausen's liquid media (Ellinghausen and McCullough, 1965) with 1 ml of semi-solid stock culture of the kidney isolate. This was incubated at 29°C for seven days, and then subcultured into a fresh tube of Ellinghausen's liquid media. The second culture was again incubated for seven days at 29°C. A sample of the challenge culture was then placed into a Petroff-Hausser bacterial counting chamber and counted using darkfield microscopy.

The challenge culture was diluted with Ellinghausen's liquid culture media in ten-fold dilutions from $10^7$ through $10^0$ organisms/ml.

Twenty-one day old hamsters (Engle Laboratory Animals, Inc.) were divided into eight groups of five. Each hamster was inoculated intraperitonially with a 1 ml dose of the challenge culture. Each of the eight groups of hamsters received a different concentration of the challenge culture ($10^7$, $10^6$, and so on, through $10^0$ organisms/ml). The $LD_{50}$ of the isolate in hamsters was determined by the Reed and Muench method as described by Davis et al. (1973).

Experimental Infections of Laboratory Rats

Fourteen albino laboratory rats, 175-200 grams in weight (Sasco, Inc.), were intraperitoneally injected with a 1 ml volume of the challenge culture (prepared exactly as that for hamsters). Seven of the laboratory rats received

$10^3$ organisms per milliliter and seven received $10^6$ organisms per milliliter. On the day of challenge, one rat from each group was euthanized, using the chloroform over-dose method as detailed previously for wild rats. Every four days thereafter, one rat from each group was euthanized. Blood was drawn from each rat via the cardiac puncture method. The laboratory rat serum was collected, diluted, and tested for leptospiral antibodies in the same manner as had the wild rat serum.

A kidney sample was taken from each of the two rats euthanized on day 24 of the test. The samples were processed and preserved for histological examination with the same procedures as were used for the wild rat kidneys.

Histological Examination of Renal Tissue

Fixed kidney samples were cut into one $cm^3$ blocks, dehydrated, cleared, infiltrated and embedded (Humason, 1972). Once embedded, each block was sectioned into five to seven micron thick ribbons.

Each section was mounted on a glass microscope slide, deparaffinized, hydrated, and stained with Warthin-Starry Silver stain (Humason, 1972). The prepared slides were viewed through a 1000X oil-immersion objective on a compound microscope. Photomicrographs of the prepared sections were taken through a compound microscope with a built-in camera.

# RESULTS AND DISCUSSION

Animal Trapping. A total of 82 animals were trapped over a five month period. Seventy-eight of these animals were wild rats, Rattus norvegicus. The four others were a muskrat, rabbit, thirteen-lined ground squirrel, and house mouse. (Table 1)

Trapping of the wild rats was most successful from mid-February to mid-July. Beyond July, the ground became so hard and dry that all of the weeds and vegetative cover died. This made it very difficult to catch any rats. The heat caused the rats to be less active and therefore less apt to be caught. The heat also decreased the survival rate of the trapped rats.

Isolation of Leptospires. Fourteen of seventy-eight wild rats (18%) were infected with leptospires. From these 14 infected wild rats, positive leptospiral isolations were made in 100% of the kidney tissues and 57% of the urine (Table 2). Probable reasons for the difference in the two percentages are as follows:

1) A high volume of urine in the bladder probably increases the chances of isolating leptospires shed from the kidneys. Some of the rats that were Leptospira carriers may not have had enough urine in the bladder for a positive isolation to be made.

TABLE 1. Animals trapped

| Specimen No. | Species | Sex | Weight(Gms.) | Length(Cm.) |
|---|---|---|---|---|
| 1 | R. norvegicus | M | 184 | 31.6 |
| 2 | R. norvegicus | M | 221 | 36.5 |
| 3 | R. norvegicus | F | 303 | 40.5 |
| 4 | R. norvegicus | F | 366 | 37.5 |
| 5 | R. norvegicus | M | 253 | 37.5 |
| 6 | O. zibethica | ND | ND | 54.0 |
| 7 | R. norvegicus | M | 292 | 41.0 |
| 8 | R. norvegicus | M | 359 | 41.0 |
| 9 | R. norvegicus | M | 310 | 37.5 |
| 10 | R. norvegicus | M | 472 | 43.5 |
| 11 | R. norvegicus | F | 369 | 40.5 |
| 12 | R. norvegicus | M | 287 | 40.0 |
| 13 | R. norvegicus | M | 52 | 22.6 |
| 14 | R. norvegicus | F | 190 | 36.0 |
| 15 | R. norvegicus | M | 217 | 34.0 |
| 16 | R. norvegicus | F | 496 | 42.0 |
| 17 | R. norvegicus | F | 229 | 36.0 |
| 18 | R. norvegicus | F | 264 | 37.0 |
| 19 | R. norvegicus | F | 216 | 37.0 |
| 20 | R. norvegicus | F | 192 | 35.0 |
| 21 | R. norvegicus | M | 40 | 20.0 |
| 22 | R. norvegicus | F | 206 | 36.5 |
| 23 | R. norvegicus | F | 370 | 41.5 |
| 24 | R. norvegicus | M | 205 | 35.0 |

TABLE 1. (Continued) Animals trapped

| Specimen No. | Species | Sex | Weight(Gms.) | Length(Cm.) |
|---|---|---|---|---|
| 25 | R. norvegicus | F | 210 | 34.5 |
| 26 | R. norvegicus | F | 312 | 41.0 |
| 27 | R. norvegicus | F | 202 | 34.5 |
| 28 | R. norvegicus | M | 334 | 38.5 |
| 29 | R. norvegicus | F | 355 | 38.5 |
| 30 | R. norvegicus | M | 143 | 32.5 |
| 31 | R. norvegicus | F | 205 | 34.5 |
| 32 | R. norvegicus | M | 394 | ND |
| 33 | R. norvegicus | F | 255 | 38.0 |
| 34 | R. norvegicus | M | 218 | 34.5 |
| 35 | R. norvegicus | F | 171 | 32.0 |
| 36 | R. norvegicus | F | 285 | 37.0 |
| 37 | R. norvegicus | F | 51 | 21.5 |
| 38 | R. norvegicus | F | 235 | 34.5 |
| 39 | R. norvegicus | F | 170 | 34.0 |
| 40 | R. norvegicus | M | 100 | 28.5 |
| 41 | R. norvegicus | F | 217 | 37.5 |
| 42 | R. norvegicus | F | 75 | 23.0 |
| 43 | R. norvegicus | F | 296 | 38.5 |
| 44 | R. norvegicus | M | 83 | 25.5 |
| 45 | R. norvegicus | F | 250 | 37.0 |
| 46 | R. norvegicus | F | 205 | ND |
| 47 | R. norvegicus | F | 368 | 39.0 |
| 48 | R. norvegicus | F | 319 | 39.0 |

TABLE 1. (Continued) Animals trapped

| Specimen No. | Species | Sex | Weight(Gms.) | Length(Cm.) |
|---|---|---|---|---|
| 49 | R. norvegicus | M | 161 | 33.0 |
| 50 | R. norvegicus | M | 183 | 35.5 |
| 51 | R. norvegicus | M | 50 | 23.0 |
| 52 | R. norvegicus | M | 312 | 38.0 |
| 53 | R. norvegicus | M | 156 | 34.0 |
| 54 | R. norvegicus | F | 146 | 32.5 |
| 55 | R. norvegicus | F | 70 | 27.0 |
| 56 | R. norvegicus | M | 256 | 36.0 |
| 57 | R. norvegicus | F | 43 | 20.0 |
| 58 | R. norvegicus | M | 43 | 19.5 |
| 59 | R. norvegicus | F | 46 | 20.0 |
| 60 | S. floridanus | ND | 171 | 20.0 |
| 61 | R. norvegicus | M | 48 | 21.0 |
| 62 | R. norvegicus | F | 41 | 21.0 |
| 63 | R. norvegicus | F | 42 | 18.0 |
| 64 | R. norvegicus | F | 41 | 22.0 |
| 65 | C. tridecemlineateus | F | 187 | 28.0 |
| 66 | Mus musculas | F | 46 | 17.5 |
| 67 | R. norvegicus | M | 84 | 26.4 |
| 68 | R. norvegicus | M | 82 | 28.0 |
| 69 | R. norvegicus | F | 84 | 28.0 |
| 70 | R. norvegicus | M | 200 | 35.0 |
| 71 | R. norvegicus | F | 450 | 41.5 |
| 72 | R. norvegicus | M | 91 | 26.5 |

TABLE 1. (Continued) Animals trapped

| Specimen No. | Species | Sex | Weight(Gms.) | Length(Cm.) |
|---|---|---|---|---|
| 73 | R. norvegicus | M | 335 | 42.0 |
| 74 | R. norvegicus | M | 75 | 26.5 |
| 75 | R. norvegicus | M | 80 | 27.0 |
| 76 | R. norvegicus | M | 270 | 39.0 |
| 77 | R. norvegicus | M | 55 | 22.0 |
| 78 | R. norvegicus | M | 242 | 36.0 |
| 79 | R. norvegicus | F | 167 | 34.0 |
| 80 | R. norvegicus | M | 283 | 39.0 |
| 81 | R. norvegicus | M | 365 | 40.0 |
| 82 | R. norvegicus | M | 235 | 37.0 |

TABLE 2. Specimen No., sex, and site of leptospiral isolations

| Specimen No. | Sex | Kidney | Urine | Blood | Brain |
|---|---|---|---|---|---|
| 7 | M | + | - | - | - |
| 8 | M | + | + | - | - |
| 9 | M | + | + | - | - |
| 10 | M | + | + | - | - |
| 12 | M | + | + | - | - |
| 26 | F | + | - | - | - |
| 29 | F | + | + | - | - |
| 32 | M | + | + | - | - |
| 46 | F | + | - | - | - |
| 47 | F | + | - | - | - |
| 48 | F | + | - | - | - |
| 52 | M | + | - | - | - |
| 56 | M | + | + | - | - |
| 81 | M | + | + | - | - |

| Total Positives | Kidney | Urine | Blood | Brain |
|---|---|---|---|---|
| 14 | 14 | 8 | 0 | 0 |

2) Urinary leptospiral antibodies have been found in leptospirosis (Vander Hoeden, 1936; Stuart, 1956). If present, urinary antibodies would decrease the number of viable leptospires found in urine. This would reduce the chance of isolating the organism from a urine specimen.

3) Babudieri (1958), showed that virulent Leptospira favor a pH of approximately seven, and that they are most vulnerable to acidic urine. According to Zueler (1935), the rat usually has a urine pH of 5.4 to 5.8.

4) Faine (1962), suggested that leptospiral isolates from the urine are slow to revert from the "renal" to the "cultured" growth state. Therefore there may have been isolates present in some of the urine which failed to show any leptospiral growth.

Leptospiral isolations were negative from the blood and brain tissues. This indicated that the rats were chronic carriers and not in the acute stage of leptospiremic infection. These results are similar to other studies (Alexander, 1974; Babudieri, 1958).

A rat 20 cm in length weighs approximately 200 gm, which is approximately the size of a wild rat when it reach es maturity. Only the mature wild rats were infected with leptospires (Table 3). Walch-Sorgdrager (1939), found the incidence of infection to be about eight times greater in rats measuring over 20 cm as that in rats shorter than 20 cm. A low infection rate in immature wild rats has been evidenced

TABLE 3. Leptospiral isolation results by weight group

| Weight (Grams) | No. Trapped | No. Positive Isolations |
|---|---|---|
| 0-50 | 8 | 0 |
| 51-100 | 13 | 0 |
| 101-150 | 3 | 0 |
| 151-200 | 9 | 0 |
| 201-250 | 15 | 1 |
| 251-300 | 10 | 3 |
| 301-350 | 8 | 4 |
| 351-400 | 9 | 5 |
| 401-450 | 1 | 0 |
| 451-500 | 2 | 1 |
| Total | 78 | 14 |

by others (Walch and Walch-Sorgdrager, 1927; Smith, 1938; Kalfayan, 1947; Babudieri, 1958; Kalli et al., 1962; Fuzi and Csoka, 1962).

All of the infected rats were caught near a hog buying station. None of the adult or immature rats caught at the zoo were carrying leptospires. This fact suggests that the incidence of infection in the Des Moines wild rat population is localized to specific areas.

None of the four non-rat specimens were carrying leptospires.

Titration of Wild Rat Sera. Table four shows the titers of each wild rat from which leptospires were isolated. No immature wild rats were carrying leptospiral antibodies. This evidence further supports the conclusions made by Birnbaum et al. (1972b), and Babudieri (1958).

The leptospiral isolates reacted only with icterohaemorrhagiae anti-serum. The wild rat sera reacted only with serotype icterohaemorrhagiae live antigen. Serotype icterohaemorrhagiae is the leptospire most commonly isolated from wild rats (Noguchi, 1917; Ido et al., 1917; Walch and Walch-Sorgdrager, 1927; Lewis, 1942; Larson, 1943; Humphreys et al., 1953; Babudieri, 1958; Broom, 1958; Vander-Hoeden, 1958; Higa and Fujianaka, 1976; Salt and Little, 1977). Serotype pomona is the leptospire most commonly isolated from swine. The wild rats trapped at the hog buying station were negative for pomona antibodies and organisms. This

TABLE 4. Microscopic agglutination test end titers of infected wild rats

| Specimen No. | Titer | Sex | Weight(Gms.) | Length(Cm.) |
|---|---|---|---|---|
| 7 | 1:100 | M | 292 | 41.0 |
| 8 | 1:80 | M | 359 | 41.0 |
| 9 | 1:1000 | M | 310 | 37.5 |
| 10 | 0 | M | 472 | 40.5 |
| 12 | 1:80 | M | 287 | 40.0 |
| 26 | ND | F | 312 | 41.0 |
| 29 | 1:80 | F* | 355 | 38.5 |
| 32 | 1:800 | M | 394 | ND |
| 46 | 0 | F | 205 | ND |
| 47 | 1:100 | F* | 368 | 39.0 |
| 48 | 0 | F* | 319 | 38.5 |
| 52 | 1:1000 | M | 312 | 38.0 |
| 56 | 1:1000 | M | 256 | 36.0 |
| 81 | 1:100 | M | 365 | 40.0 |

(*pregnant)

TABLE 5. Virulence titration of leptospires from specimen No. 7

| Organism Dilution | Mortality Ratio | Number Dead | Number Survivors | Total Dead | Total Survivors | Mortality Ratio | Mortality Percentage |
|---|---|---|---|---|---|---|---|
| 10-1 | 5/5 | 5 | 0 | 24 | 0 | 24/24 | 100% |
| 10-2 | 5/5 | 5 | 0 | 19 | 0 | 19/19 | 100% |
| 10-3 | 5/5 | 5 | 0 | 14 | 0 | 14/14 | 100% |
| 10-4 | 5/5 | 5 | 0 | 9 | 0 | 9/9 | 100% |
| 10-5 | 3/5 | 3 | 2 | 4 | 2 | 4/6 | 67% |
| 10-6 | 1/5 | 1 | 4 | 1 | 6 | 1/7 | 14% |
| 10-7 | 0/5 | 0 | 5 | 0 | 11 | 0/11 | 0% |
| 10-8 | 0/5 | 0 | 5 | 0 | 16 | 0/16 | 0% |

$$LD_{50} = \frac{\text{(\% Mortality at dilution above 50\%) - 50\%}}{\text{(\% Mortality at dilution above 50\%) - (\% Mortality at dilution below 50\%)}}$$

$$\frac{67 - 50}{67 - 14} = 0.32 = \text{proportionate distance}$$

Negative log of lower dilution (above 50%) - (proportionate distance) X = dilution factor (log 10)

$$\begin{array}{r} -5.00 \\ \underline{-0.32} \\ -5.32 \end{array} = \text{Log } LD_{50} \text{ titer/ml}$$

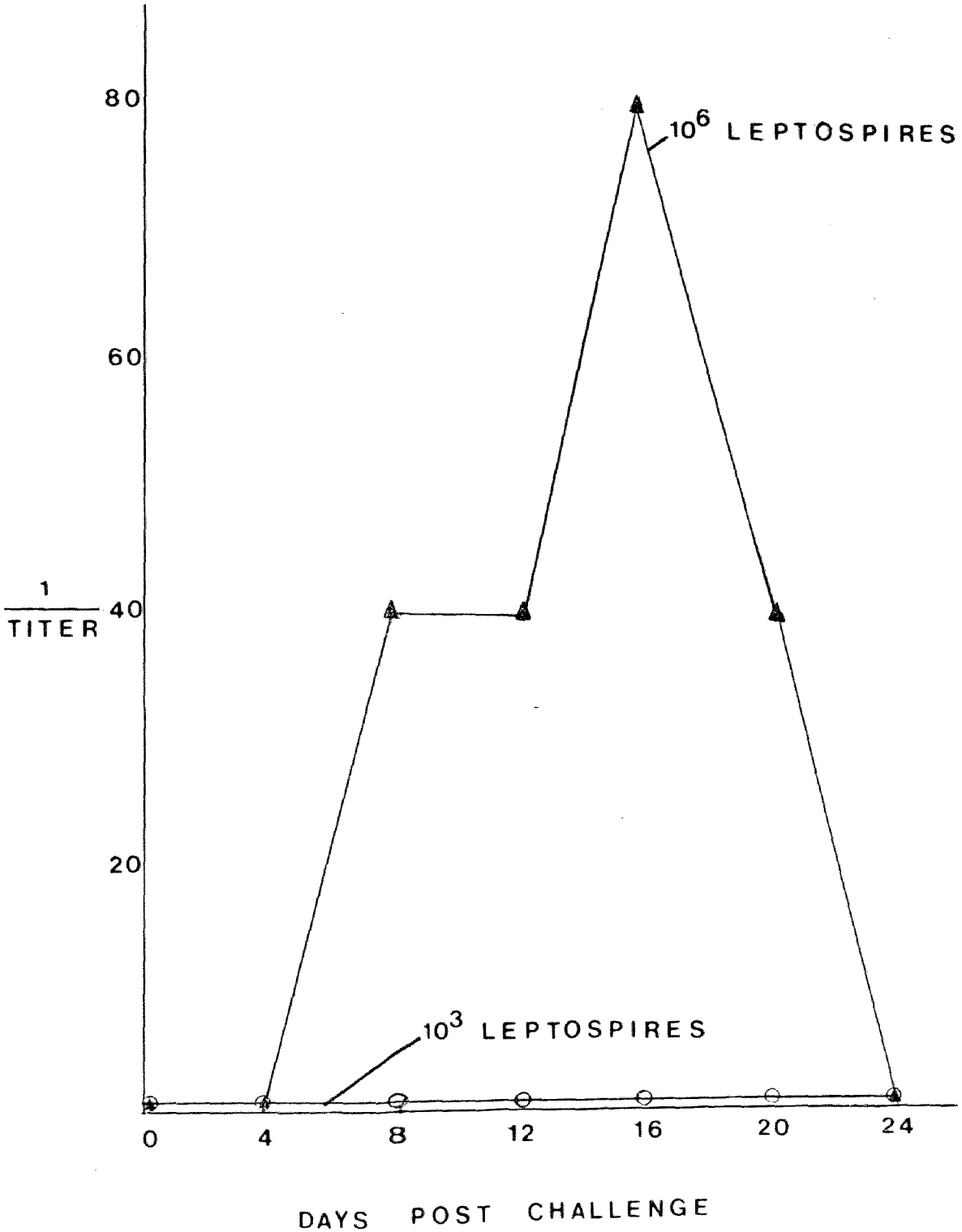
$LD_{50} = 10 - 5.32$/ml

indicates that serotype pomona is host specific or there were not any infected swine.

Virulence of Wild Isolate in Hamsters. An isolate from the kidney of a wild male rat weighing 292 grams and 41 cm in length was titrated for hamster virulence. Table five shows the calculations done to determine that the $LD_{50}$ of the Leptospira isolate from the wild rat was $10^{5.3}$/ml in hamsters.

Experimental Infection of Laboratory Rats. Figure one shows the antibody response illicited by the young (under 200 grams) laboratory rats in response to the challenge of the wild rat kidney isolate. The lab rats that received $10^3$ leptospires did not respond serologically (no antibody) titer). One lab rat receiving $10^6$ leptospires responded with a 1:80 rise in antibody titer against the challenge. The two concentrations of leptospires were chosen because they represented challenge doses above and below the lethal dose fifty for hamsters. Neither group of rats showed any clinical signs of illness. The lethal dose fifty of the wild isolate had no observable effect on the young lab rats. Kalli et al. (1962), studied several albino laboratory rat colonies infected with L. ictero. Their study revealed that immature rats (4 months old or younger) were all Leptospira negative. They concluded that this was due to passive (maternal) immunity. Fuzi and Csoka (1962), also found the incidence of infection very low in immature laboratory rats.

Figure 1. Serum antibody levels of laboratory rats challenged with the leptospiral isolate from specimen No. 7.


80
60
1/TITER 40
20
$10^6$ LEPTOSPIRES
$10^3$ LEPTOSPIRES
0
4
8
12
16
20
24
DAYS POST CHALLENGE

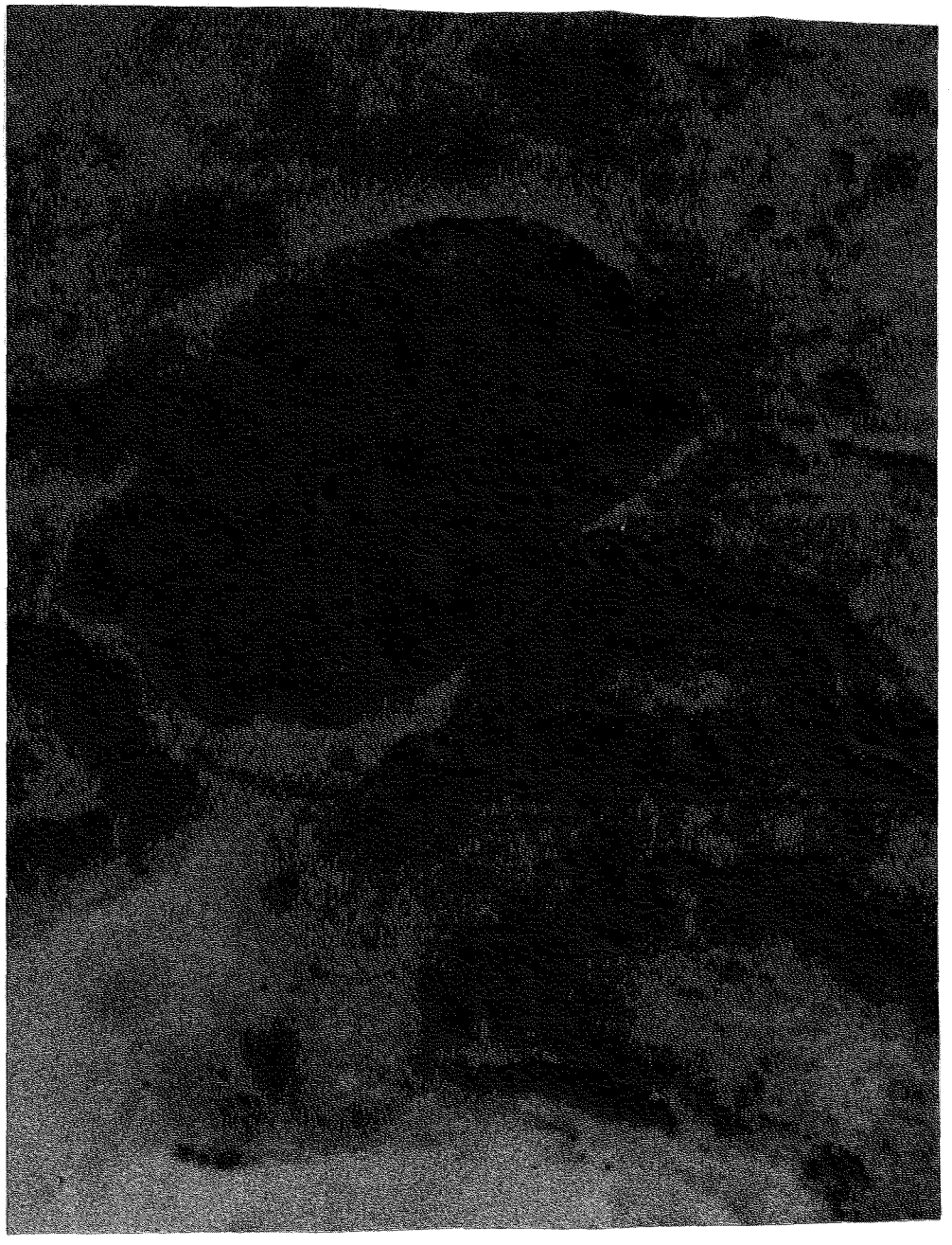


Figure 2. Photomicrograph of a kidney section of specimen No. 81 taken through a microscope at 1000X magnification. The Leptospira are stained dark brown to black, and can be seen nestled away in nephridic tissue.

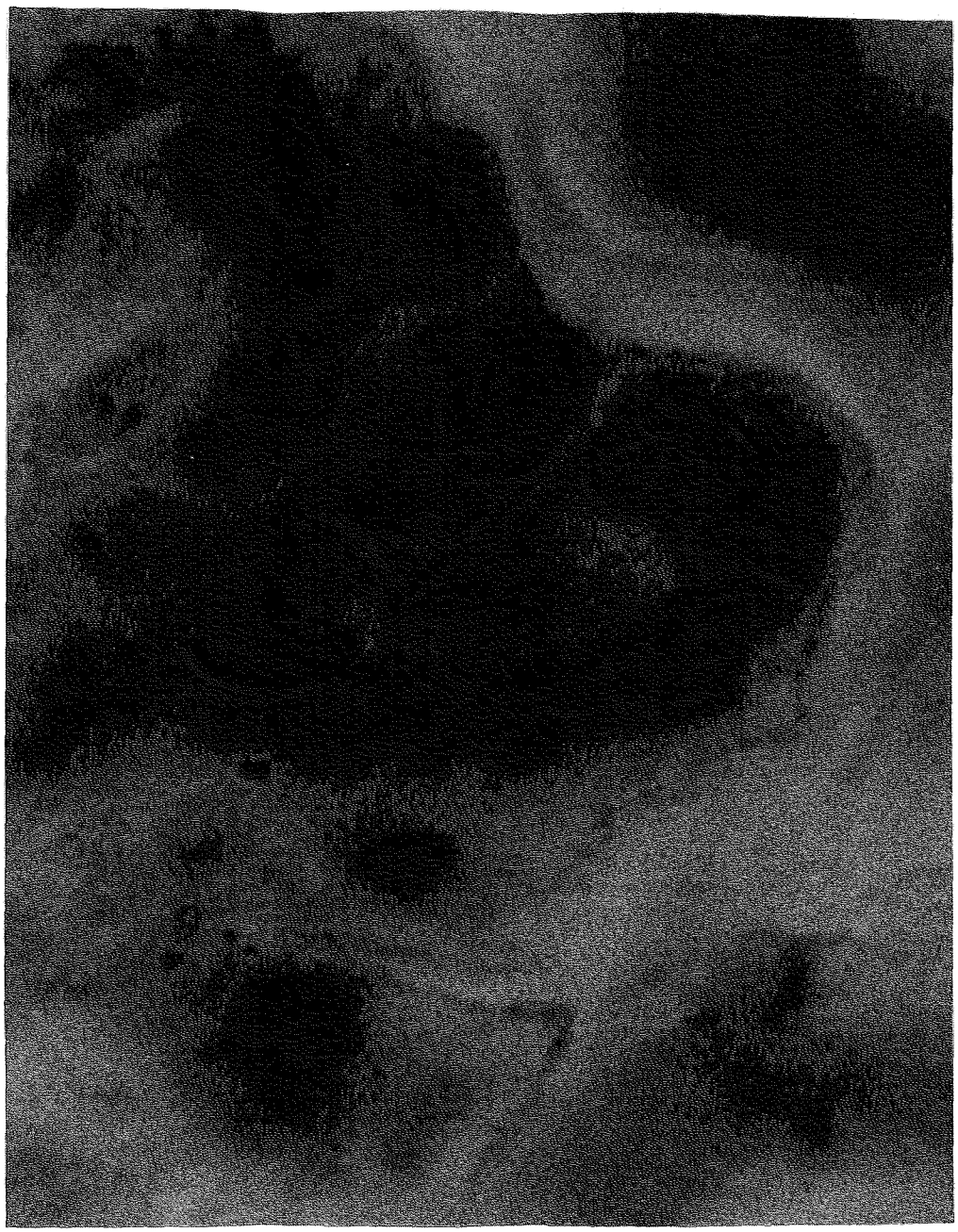


Figure 3. Photomicrograph of a kidney section of specimen No. 81 taken through a microscope at 2000X magnification. The Leptospira are stained dark brown to black, and can be seen nestled away in the nephridic tissue.

Histological Examination of Renal Tissue. Upon microscopic examination, numerous Leptospira were seen nesting in the lumen of the nephridic tubules of an infected wild rat kidney. Figures two and three show photomicrographs of L. icterohaemorrhagiae in the kidney of specimen No. 81. Electron microscopic studies of the relationship between leptospires and renal tissue have shown that in the chronic-shedder stage of infection, most leptospires are found in the lumen of the proximal tubules (Miller and Wilson, 1967).

Microscopic examination of the laboratory rat kidneys were negative for Leptospira. The inoculated leptospires apparently never reached the renal tissues of the immature laboratory rats. The leptospires were either inactivated via maternal antibody protection (Birnbaum et al., 1972b), or rapidly phagocytized by the reticulo-endothelial system (Faine, 1964).

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